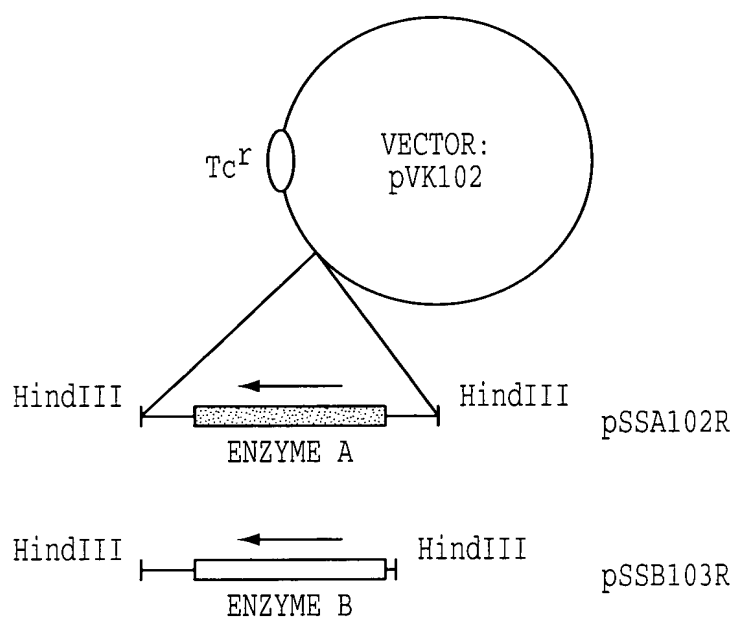




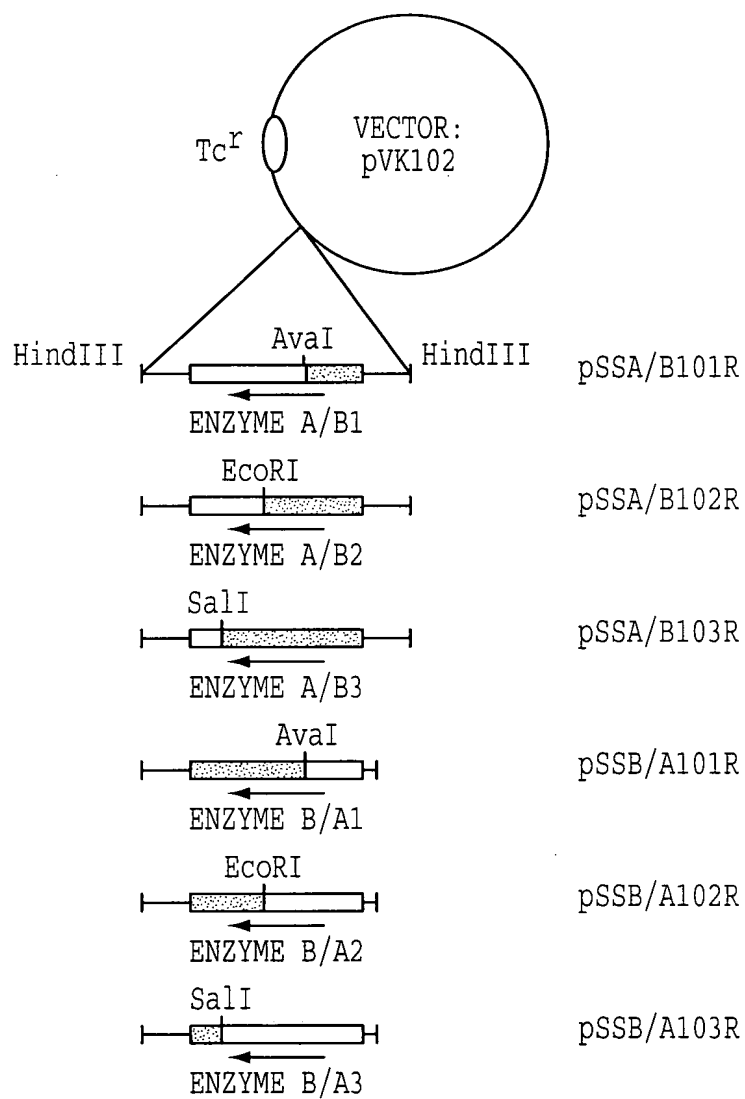
In re Application of: Akira ASAKURA, *et al.*  
Serial No.: 09/470,667  
For: **NOVEL ALCOHOL/ALDEHYDE  
DEHYDROGENASES**



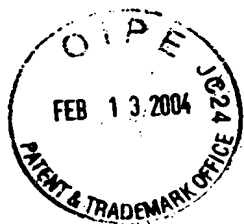
**FIG. 1**



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DEHYDROGENASES**

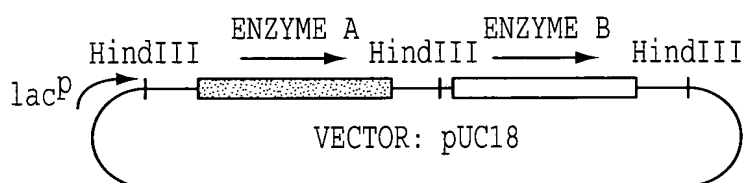


**FIG. 2**



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DEHYDROGENASES**

pSSAB201



pSSBA201

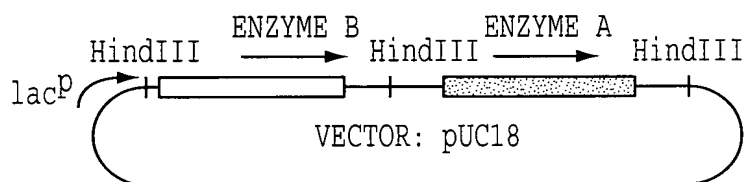
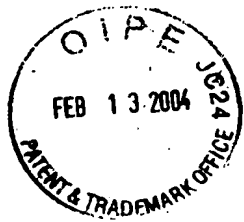
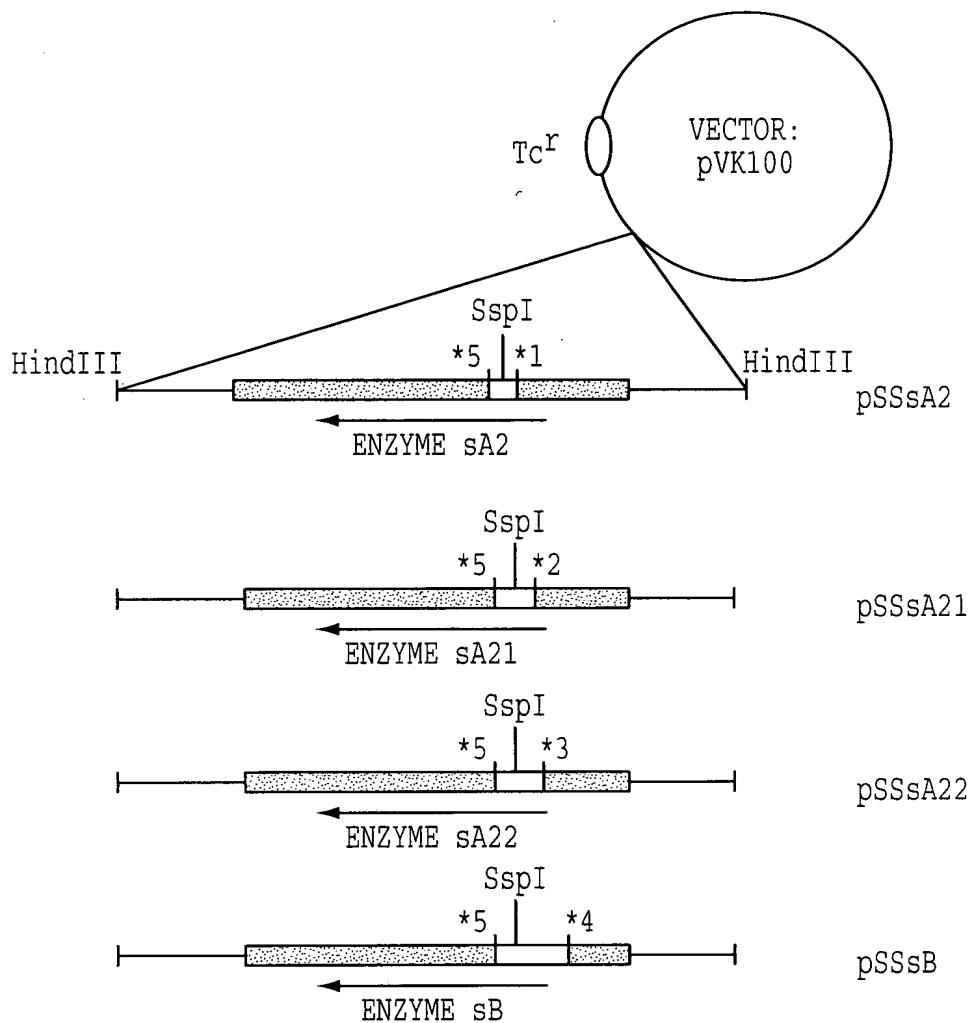


FIG. 3



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 Serial No.: 09/470,667  
 For: **NOVEL ALCOHOL/ALDEHYDE  
 DEHYDROGENASES.**



#### RECOMBINATION SITE

- \*1 : AMINO ACID RESIDUE NO. 135 OF MATURE ENZYME A
- \*2 : AMINO ACID RESIDUE NO. 128 OF MATURE ENZYME A
- \*3 : AMINO ACID RESIDUE NO. 125 OF MATURE ENZYME A
- \*4 : AMINO ACID RESIDUE NO. 95 OF MATURE ENZYME A
- \*5 : AMINO ACID RESIDUE NO. 180 OF MATURE ENZYME B,  
 WHICH NUCLEOTIDE SEQUENCE OF *Ava*I SITE ENCODES

**FIG. 4**



In re Application of: Akira ASAKURA, et al.  
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 DEHYDROGENASES

ENZYME A 1 : QVTPVTDELL ANPPAGEWIS YGQNQENYRH SPLTQITTEN VGQLQLVWAR GMQPGKVQVT  
 \*\*\*\*\*  
 ENZYME B 1 : QVTPITDELL ANPPAGEWIN YGRNQENYRH SPLTQITADN VGQLQLVWAR GMEAGAVQVT

61 : PLIHDGVMYL ANPGDVIQAI DAKTGDLIWE HRRQLPNIAT LNSFGEPTRG MALYGTNVYF  
 \* \*\*\*\*\*  
 61 : PMIHDGVMYL ANPGDVIQAL DAQTGDLIWE HRRQLPAVAT LNAQGDRKRG VALYGTSLYF

AvaI

121 : VSWDNHLVAL DTATGQVTFD VDRGQGED-M VSNSSGPIVA NGVIVAGSTC QYSPFGCFVS  
 \*\*\*\*\*  
 121 : SSWDNHLIAL DMETGQVVFD VERGSGEDGL TSNTTGPIVA NGVIVAGSTC QYSPYGC FIS

180 : GHDSATGEEL WRNYFIPRAG EEGDETWGND YEARWMTGAW GQITYDPVTN LVHYGSTAVG  
 \*\*\*\*\*  
 181 : GHDSATGEEL WRNHFIPQPG EEGDETWGND FEARWMTGVW GQITYDPVTN LVFYGSTGVG

240 : PASETQRGTP GGTLYGTNTR FAVRPDTGEI VWRHQTLPRD NWDQECTFEM MVTNVDVQPS  
 \*\*\*\*\*  
 241 : PASETQRGTP GGTLYGTNTR FAVRPDTGEI VWRHQTLPRD NWDQECTFEM MVANVDVQPS

EcoRI

300 : TEMEGLQSIN PNAATGERRV LTGVPCKTGT MWQFDAETGE FLWARDTNYQ NMIESIDENG  
 \*\*\*\*\*  
 301 : AEMEGLRAIN PNAATGERRV LTGAPCKTGT MWSFDAASGE FLWARDTNYT NMIASIDETG

360 : IVTVNEDAIL KELDVEYDVC PTFLGGRDWP SAALNPDSGI YFIPLNNVCY DMMAVDQEFT  
 \*\*\*\*\*  
 361 : LVTVNEDAVL KELDVEYDVC PTFLGGRDWS SAALNPDTGI YFLPLNNACY DIMAVDQEFS

Sali

420 : SMDVYNTSNV TKLPPGKDMI GRIDAIDIST GRTLWSVERA AANYSPVLST GGGVLFNGGT  
 \*\*\*\*\*  
 421 : ALDVYNTSAT AKLAPGFENM GRIDAIDIST GRTLWSAERP AANYSPVLST AGGVVFNNGGT

480 : DRYFRALSQE TGETLWQTRL ATVASGQAIS YEVDGMQYVA IAGGGVSYGS GLNSALAGER  
 \*\*\*\*\*  
 481 : DRYFRALSQE TGETLWQARL ATVATGQAIS YELDGVQYIA IGAGGLTYGT QLNAPLA-EA

540 : VDSTAIGNAV YVFALPQ  
 \*\*\*  
 540 : IDSTSVGNAI YVFALPQ

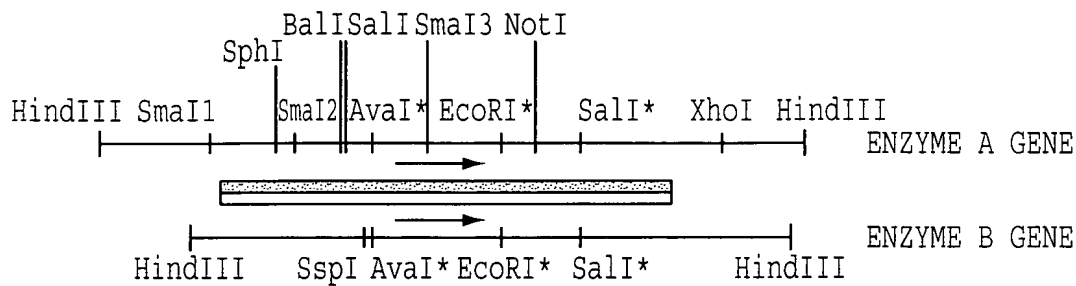
\* : NUCLEOTIDE SEQUENCES ENCODING THESE REGIONS ARE THE RESTRICTION SITES  
 FOR AvaI, EcoRI, AND Sali WHICH WERE USED FOR CONSTRUCTING CHIMERA  
 GENES SHOWN IN FIG. 2.

FIG. 5



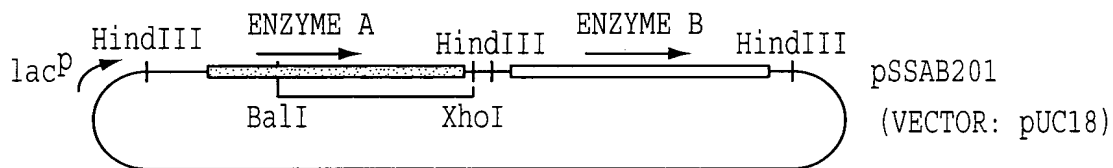


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DEHYDROGENASES**

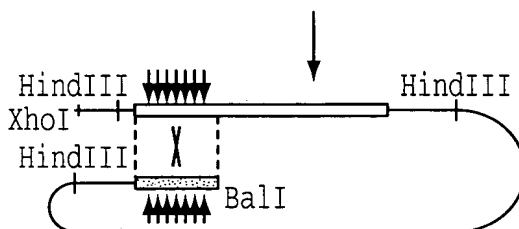


\*: AvaI, EcoRI, SalI SITES USED FOR CONSTRUCTING CHIMERA GENES  
SHOWN IN FIG. 2 AND 6.

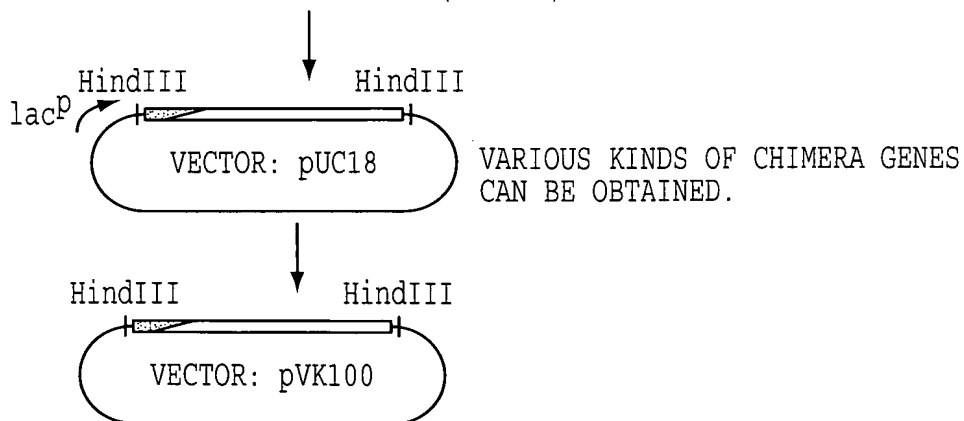
**FIG. 7**



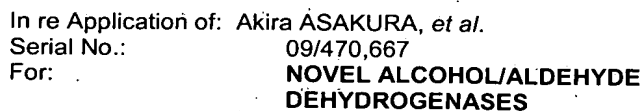
LINEARIZATION WITH XhoI AND BalI



TRANSFORM *E. coli* JM101 (*rec A*+)



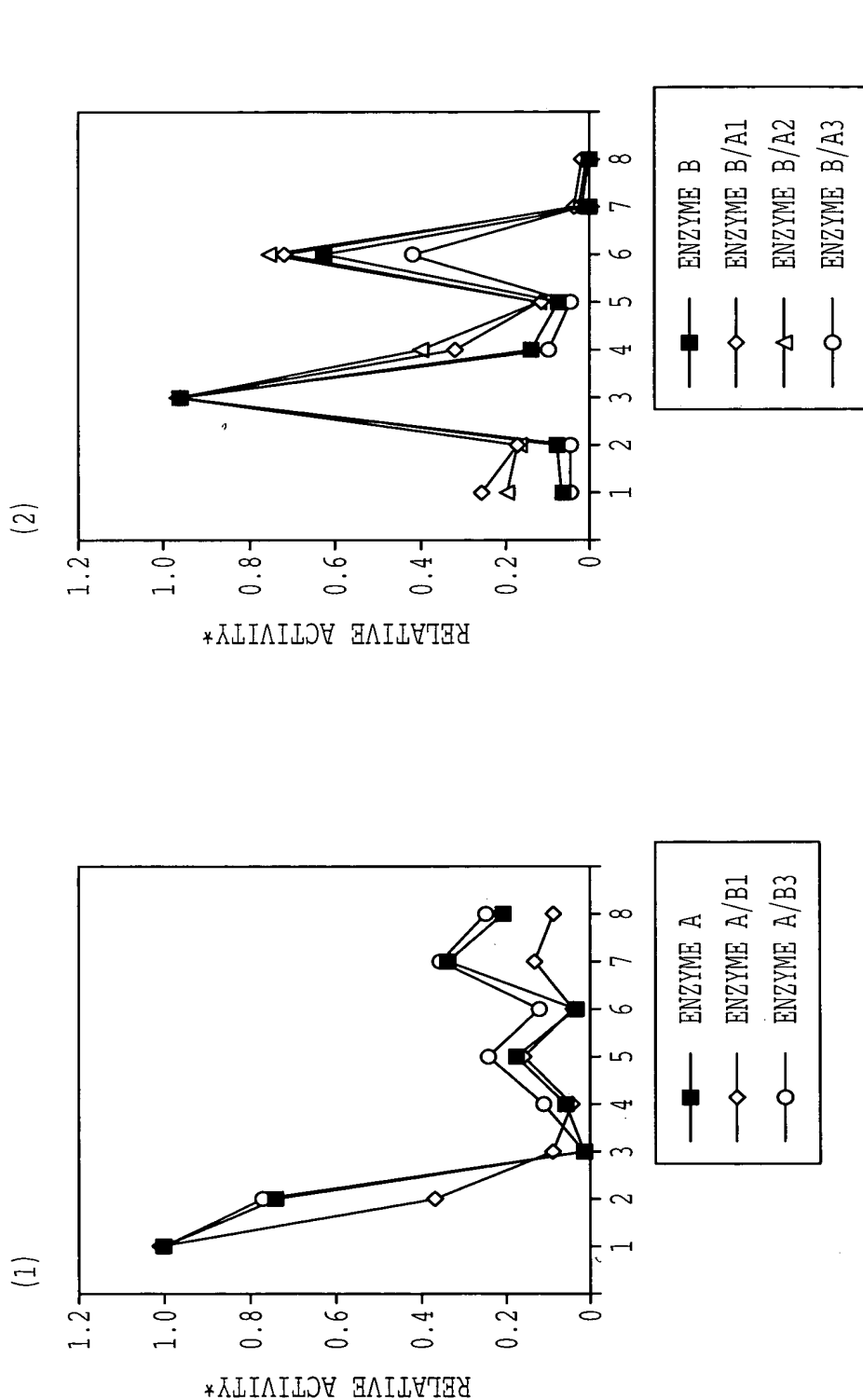
**FIG. 8**







In re Application of: Akira ASAKURA, *et al.*  
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DEHYDROGENASES



1. n-PROPANOL, 2. ISOPROPANOL, 3. D-GLUCOSE, 4. L-SORBOSE  
5. D-SORBITOL, 6. D-MANNITOL, 7. L-SORBOSE, 8. D-FRUCTOSE

\*ENZYME ACTIVITY WAS NORMALIZED RELATIVE TO ACTIVITY FOR n-PROPANOL (1), OR D-GLUCOSE (2).  
ENZYME A/B2 WAS EXCEPTED BECAUSE OF ITS LOW EXPRESSION IN *P. putida*.

FIG. 11